Immature and mature antibodies as defenders against cancer

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Antibodies – unmatured and matured defenders against cancer

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Antibodies (Abs) are glycoprotein molecules which represent an essential component of the adaptative immune response. They are secreted in body fluids and act as a critical part of the immune defense. Although recognition of self-antigens by Abs is normally counter-selected during the ontogeny of B cells, autoreactive Abs can be generated in result of pathological immune response as observed in autoimmune diseases. Autoantibodies can also be produced in cancer patients where they may have beneficial roles.[1] Indeed, the presence of intratumoral antibody-secreting cells (ASCs) and B-cell-rich tertiary lymphoid structures (TLSs) have been observed in many cancer types and generally correlate with a favorable clinical prognosis.[2-6] The existence of specific anti-tumor antibody responses suggests that tumor cells express molecules that are recognized as non-self antigens by immune system or that the tolerance to self-antigens expressed on cancer cells is lost.[1,4] The study of the origin of tumor-reactive Abs, their specificity and functions are of paramount importance to figure out the development of adaptive anti-tumor immune response and to find novel therapeutic avenues. A recent publication in *Cell* by Mazor et al.[7] has provided an important advance in our understanding about antibody responses in cancer. Particularly, this work paves the way for distinction between antibody-reactive tumors versus antibody non-reactive tumors, that is reminiscent with the concept of non-infiltrated "cold" tumor microenvironment (TME) in opposition with highly-infiltrated "hot" TME.[8] Moreover, an important finding of this study is the demonstration that tumor-directed autoreactivity may be both pre-existing or induced by somatic hypermutations, a mechanism related to the chronicity of cancer disease.

In their study, Mazor et al.[7] examined tumor samples from untreated high grade serious ovarian cancer (HGSOC) patients. The samples were stained for IgG Abs and by using a machine learning approach, the localization of IgG was analyzed by histology. Data showed that many of HGSOC primary tumors had deposit of endogenous IgG molecules at levels which are much

higher than the level of background IgG deposits detected in healthy ovarian tissue (64 vs 10%, respectively). The authors further confirmed this observation on live epithelial and metastatic cells using flow cytometry. Furthermore, after purification of polyclonal IgGs from tumor ascites, the binding of Abs to cancer cells was studied and shown that 72% of ascites contained IgG specific for tumor cells. Importantly, using immunohistochemistry and flow cytometry analysis, the authors revealed that tumor samples present tertiary lymphoid structures (TLS)-like clusters with more than half of the intratumoral ASCs producing IgG1 Abs. These results imply that endogenous tumor-reactive Abs, probably produced by intratumoral ASCs, can bind the surface of ovarian cancer cells. The binding of endogenous Ab to tumor cells might be a frequent feature of anti-tumoral immune responses because it was also found in several other types of cancers tested in the present study and other studies, particularly in renal and urothelial carcinoma.[6,7] Importantly, Mazor et al. demonstrated that the presence of IgG producing ASC and deposits of IgG on the surface of tumor cells was associated with a significantly better prognosis for patients with HGSOC, these findings being in line with the positive impact of the presence of B-cell containing TLS observed in several cancers.[4-6,9,10]

An essential aspect of the work of Mazor et al. is the investigation of the origin of anti-tumor specificity of autoreactive B cells infiltrating the tumor. After a single cell sorting from fresh tumor samples, the authors sequenced the genes encoding V_H and V_L domains of infiltrating $IgG1^+$ ASC. They found that sequences encoding the variable domains in these cells present a high level of somatic hyper mutations (SHM) that is in the same range as the one documented for human memory B cells. Moreover, the reconstruction of cell lineages of the intratumoral ASCs showed high degree of clonal relationship, suggesting that a considerable portion of intratumoral B cells originate from a few common ancestors.

In a next stage of the study the authors cloned from intratumoral ASC and expressed a repertoire of recombinant IgG1 Abs. This repertoire contained representative Abs from

different clonal families and it was used for mechanistic and functional studies. Importantly, a significant fraction of the recombinant Abs (15/27) demonstrated binding reactivity towards primary ovarian cancer cell cultures. To assess the impact of SHM on anti-tumor Ab specificity, the authors reverted the sequences encoding V_H and V_L domains into their germline versions and they observed, using immunofluorescence assays, that half of the tumor-reactive Abs lost their binding capacity to cancer cells after reversion to germline. This result suggests that anti-tumor specificity of Abs in patients with ovarian cancer is both natural (germline encoded) and acquired as a consequence of affinity maturation and selection of B cells. The generation of novel autoreactivities of Abs as a result of insertion of SHM into immunoglobulin genes was described before.[11] Autoreactive Abs detected in cancer patients traditionally come from self-reactive B cells that have escaped the tolerance mechanisms that occur during the B cell ontogeny.[1,4] Acquisition of SHM typically takes place in germinal centers that can be located in lymph nodes as well as in intratumoral tertiary lymphoid structures. The latter have been associated to generation of intratumoral ASCs producing Abs directed towards tumor-associated antigens in various cancers.[4] Thus, we can conclude that in addition to naturally autoreactive Abs that are specific to the tumor antigen, Abs that do not bind a self-target tumor antigen in their original germline version may be positively selected during B cell maturation for their newly acquired tumor specificity.

In addition to affinity matured Abs, unmatured Abs with germline configuration of their V genes have been demonstrated to play an important role in anti-tumor defense in animal models. Thus, several recent studies revealed that natural IgM Abs contribute for tumor elimination in mice.[12-14] These studies showed that the presence of neoantigens on cell surface results in a binding of natural IgM Abs, a process that triggers elimination of the modified cells. In contrast to Abs identified in the study of Mazor et al., the natural IgM Abs are frequently polyreactive. Hence they can have distinct mode of action in protection against cancer. It was

proposed that these natural auto-reactive Abs may increase the immunogenicity of tumor cells by allowing antigen-presenting cells to present cognate tumor antigens to specific T cells.[14] It remains to be clarified whether in humans the Abs with germline configuration and specificity for tumor antigens exert similar anti-tumoral effect and also whether they can trigger the loss of self-tolerance and generation of tumor-reactive autoantibodies following SHM process as observed in the study of Mazor et al.

Further, Mazor et al. used the repertoire of recombinant Abs to identify the target antigens on cancer cell. The authors identify the matrix metalloproteinase 14 (MMP14) as a major target for the patient-derived Abs. They observed a milder cross-reactivity to other metalloproteinases, but they did not detect reactivities to other human antigens. It is noteworthy that MMP14 is overexpressed by tumors and contributes along with other metalloproteinases for remodeling of extracellular matrix and tumor invasion.

Finally, Mazor et al. studied the Fc-mediated functions of recombinant Abs as well as of polyclonal IgG isolated from tumor ascites. It was found that polyclonal Abs and some of the recombinant Abs were efficient to induce antibody-dependant cellular cytotoxicity (ADCC) by NK cells and antibody-mediated cell phagocytosis (ADCP) by monocytes. Although these Abs bind efficiently to tumor cells, cytotoxic effector cells might be rare in HGSOC patient tumors and their recruitment in the tumor microenvironment can be difficult. Therefore, it remains unclear if the beneficial effect on patient survival of the presence of IgG in the tumor is mediated through activation of effector functions by anti-tumoral Abs or they exert effect through other mechanisms. In this respect, it will be interesting to be elucidated what are the functional consequences of Ab binding on the enzyme activity of metalloproteinase. If Abs inhibit the remodeling of extracellular matrix, this suggests that Mazor and colleagues identified a putative therapeutic target for cancer therapy with Abs. Moreover, it is important

to understand whether the metalloproteinases are typical target for humoral responses against other types of cancer or they are specific only for HGSOC.

In conclusion the study of Mazor and al. represents an important step in understanding antibody responses against cancer. This study clearly highlights that there is a specific selection and affinity maturation of high affinity autoreactive B cells targeting tumor antigens (**Figure 1**). In view of the fact that mAbs produced in tumor microenvironment are directed against self-non-mutated proteins rather than being restricted to tumor-specific antigens, this study highlights the close relationship between self-tolerance breakdown and anti-tumor immunity. *In situ* high expression of particular self-antigens (e.g., in ovarian and pancreatic cancers which are highly fibrotic, a protein involved in the remodeling of the extracellular matrix) combined with pro-inflammatory environment might promote tolerance breakdown. Interestingly, although Mazor et al. report in this study that self-reactive antibodies formed in cancer patients have the potential to bind healthy tissues in addition to tumor cells, no signs of autoimmunity have been detected. Moreover, large-scale retrospective study of patients with ovarian cancer over a follow-up period of up to 15 years did not reveal increased incidence of autoimmune diseases, raising questions about the relation between autoreactivity and autoimmune manifestations. The non-persistence over the long term of sera autoantibodies produced in cancer patients and the deleterious impact of certain therapies such as chemotherapy on the production of antibodies by B lymphocytes are among the hypotheses given by the authors to explain this absence of autoimmunity occurrence. This issue is different in patients treated with anti-PD-1 and/or anti-CTLA-4 mAbs, since these treatments, on the contrary, probably potentiate the reactivation of autoreactive (probably also anti-tumor) B-cell clones, promoting the development of various autoimmune side effects in treated patients.[15]

The study opens numerous questions. By addressing these questions, important fundamental insights and novel therapeutic approaches can be gained.

References

1 Zaenker, P., Gray, E. S. & Ziman, M. R. Autoantibody Production in Cancer--The Humoral Immune Response toward Autologous Antigens in Cancer Patients. *Autoimmun Rev* **15**, 477-483 (2016).

2 Dieu-Nosjean, M. C. *et al.* Tertiary lymphoid structures, drivers of the anti-tumor responses in human cancers. *Immunol Rev* **271**, 260-275 (2016).

3 Teillaud, J. L. & Dieu-Nosjean, M. C. Tertiary Lymphoid Structures: An Anti-tumor School for Adaptive Immune Cells and an Antibody Factory to Fight Cancer? *Front Immunol* **8**, 830 (2017).

4 Sharonov, G. V., Serebrovskaya, E. O., Yuzhakova, D. V., Britanova, O. V. & Chudakov, D. M. B cells, plasma cells and antibody repertoires in the tumour microenvironment. *Nat Rev Immunol* **20**, 294-307 (2020).

5 Petitprez, F. *et al.* B cells are associated with survival and immunotherapy response in sarcoma. *Nature* **577**, 556-560 (2020).

6 Meylan, M. *et al.* Tertiary lymphoid structures generate and propagate anti-tumor antibody-producing plasma cells in renal cell cancer. *Immunity* **55**, 527-541 e525 (2022).

7 Mazor, R. D. *et al.* Tumor-reactive antibodies evolve from non-binding and autoreactive precursors. *Cell* **185**, 1208-1222 e1221 (2022).

8 Galon, J. & Bruni, D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. *Nat Rev Drug Discov* **18**, 197-218 (2019).

9 Germain, C. *et al.* Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. *Am J Respir Crit Care Med* **189**, 832-844 (2014).

10 Kroeger, D. R., Milne, K. & Nelson, B. H. Tumor-Infiltrating Plasma Cells Are Associated with Tertiary Lymphoid Structures, Cytolytic T-Cell Responses, and Superior Prognosis in Ovarian Cancer. *Clin Cancer Res* **22**, 3005-3015 (2016).

11 Tiller, T. *et al.* Autoreactivity in human IgG+ memory B cells. *Immunity* **26**, 205-213 (2007).

12 Atif, S. M. *et al.* Immune Surveillance by Natural IgM Is Required for Early Neoantigen Recognition and Initiation of Adaptive Immunity. *Am J Respir Cell Mol Biol* **59**, 580-591 (2018).

13 Rawat, K., Tewari, A., Morrisson, M. J., Wager, T. D. & Jakubzick, C. V. Redefining innate natural antibodies as important contributors to anti-tumor immunity. *Elife* **10** (2021).

14 Rawat, K. *et al.* Natural Antibodies Alert the Adaptive Immune System of the Presence of Transformed Cells in Early Tumorigenesis. *J Immunol* **209**, 1252-1259 (2022).

15 Hu, W., Wang, G., Wang, Y., Riese, M. J. & You, M. Uncoupling Therapeutic Efficacy from Immune-Related Adverse Events in Immune Checkpoint Blockade. *iScience* **23**, 101580 (2020).

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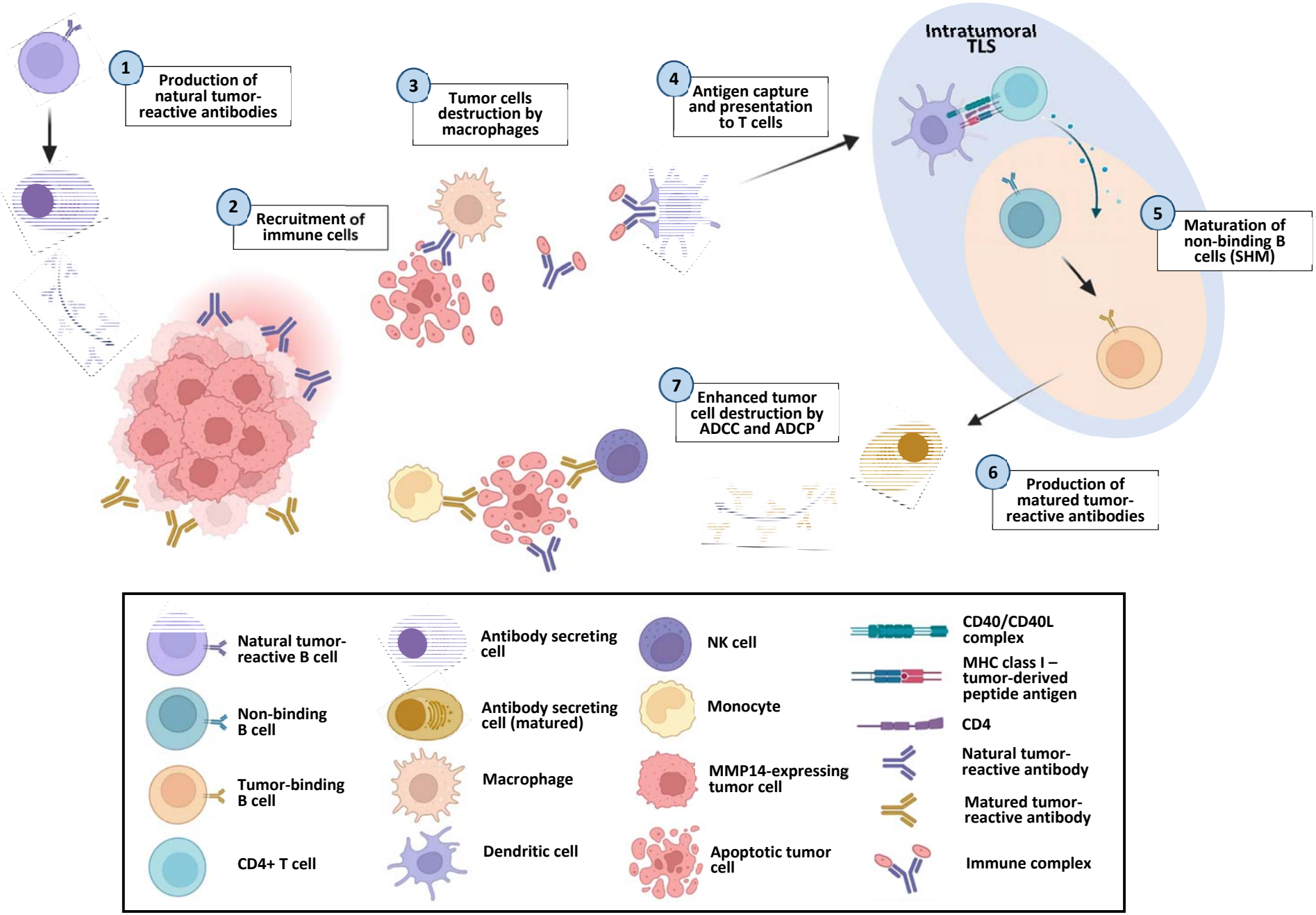
Figure legend.

Role of unmatured and matured antibodies in anti-cancer immune defense

Hypothetical overview of the development of a humoral immune response directed against the tumor. Natural germline-encoded tumor-binding Abs can recognize overexpressed antigen or neoantigen on the tumor cell surface at early stage of tumorigenesis (1). These Abs increase the immunogenicity of tumor cells (2), which leads to tumor cell destruction and/or to the recruitment of immune cells such as antigen-presenting cells (3,4). Then, activation of tumor-specific T cells in intratumoral TLS allows B cells that do not recognize these antigens to enter the maturation process and acquire specificity to tumor antigen through SHM (5). Finally, matured B cells differentiate into ASCs that produce another type of tumor-reactive Abs (6) and participate in the humoral anti-tumor immune response through antibody-dependent mechanisms (ADCC, ADCP) (7). Figure was prepared using BioRender (https://biorender.com/).


1
Production of natural tumor-reactive antibodies
2
Recruitment of immune cells
3
Tumor cells destruction by macrophages
4
Antigen capture and presentation to T cells
Intratumoral TLS
5
Maturation of non-binding B cells (SHM)
6
Production of matured tumor-reactive antibodies
7
Enhanced tumor cell destruction by ADCC and ADCP
Natural tumor-reactive B cell
Non-binding B cell
Tumor-binding B cell
CD4+ T cell
Antibody secreting cell
Antibody secreting cell (matured)
Macrophage
Dendritic cell
NK cell
Monocyte
MMP14-expressing tumor cell
Apoptotic tumor cell
CD40/CD40L complex
MHC class I – tumor-derived peptide antigen
CD4
Natural tumor-reactive antibody
Matured tumor-reactive antibody
Immune complex